



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C., 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

DATE: June 6, 2019

SUBJECT: Review of Section G for MosquitoMate, Inc. Amendment to an Experimental Use Permit 89668-EUP-3 to Test *Wolbachia pipientis* wAlbB-Strain Infected *Aedes aegypti* Mosquitoes
MRID# 50860901; Decision #544680; Submission # 1035393

FROM: Amanda A. Pierce, Ph.D., Biologist
Emerging Technologies Branch
Biopesticides and Pollution Prevention Division

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THROUGH: Eric Bohnenblust, Ph.D., Senior Biologist
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TO: Wiebke T. Striegel, Ph.D., Biologist
Emerging Technologies Branch
Biopesticides and Pollution Prevention Division

ACTION REQUESTED

Review of the experimental protocol and acreage request for MosquitoMate, Inc. to amend EPA Reg. No. 89668-EUP-3.

SUMMARY

For the past three years, MosquitoMate, Inc investigated the pesticidal efficacy of a *Wolbachia pipientis* microbial pesticide for the suppression of an *Ae. aegypti* mosquito population. MosquitoMate, Inc. seeks to amend the existing EUP, 89968-EUP-3, to increase the acreage and site locations for wAlbB-strain *Ae. aegypti* testing. The request is 49,100 acres for 2019 and 2020 and the site locations include California, Texas, Puerto Rico, and the U.S. Virgin Islands (Table 1).

The agency received a resubmitted application (5/16/19), which was updated to address shortcomings in the experimental protocol and justification in response to a 75-day deficiency letter (4/25/19). The following review is based on the resubmitted application.

CONCLUSIONS

BPPD has reviewed the experimental protocol (Section G) submitted with the application and determined the protocol to be **acceptable** pending the following changes:

- The amount of control acreage in Puerto Rico in 2020 is reduced to 2,000 acres.
- The amount of control acreage in the US Virgin Islands in 2020 is reduced to 2,000 acres.
- Replace sentence “When appropriate, additional analyses may be used to gain additional power...” with a sentence indicating that parametric analyses will be used when analyzing normally distributed data as described in MosquitoMate Inc’s initial response dated May 1, 2019 to the 75-day deficiencies letter.

With these reductions, the overall approved acreage amounts to 40,100 acres (Table 2). Although this is significantly higher acreage than the previously granted 89688-EUP-3, which requested 17,660 acres for WB1 *Ae. aegypti* testing, the number of mosquitoes released is significantly fewer. Specifically, the number of proposed mosquitoes to be released in this EUP iteration is 425,700,000 males over a two-year period compared to 681,600,000 males in the previously granted EUP. This reduction in mosquitoes despite increased requested acreage is due to updates in the applicant’s experimental design. The proposed experimental design will allow the applicant to gather data relevant for a Section 3 registration by 1) testing the efficacy of WB1 male mosquitoes in diverse ecological habitats and 2) testing the hypothesis that larger treatment areas will mitigate the impact of migrant females, thereby allowing for a reduction in the overall number of WB1 males released.

BACKGROUND

The target pest is the mosquito species *Aedes aegypti*, also known as the yellow fever mosquito. The applicant, MosquitoMate, Inc., infected *Ae. aegypti* mosquitoes with the bacterium *W. pipientis*, wAlbB strain. To achieve mosquito population suppression, live male wAlbB-infected *Ae. aegypti* are released into the environment where they mate with females of the existing, native population which are not infected with the wAlbB-strain. When wAlbB-strain infected males mate with uninfected wild females, viable offspring are not produced. Therefore, by reducing the number of viable matings through continuous release of infected males, the local *Ae. aegypti* population is predicted to decline. Importantly, male mosquitoes do not bite and therefore do not transmit diseases.

TEST LOCATIONS AND ACRES

MosquitoMate proposes to test wAlbB-strain infected *Ae. aegypti* mosquitoes in: California (20,000 acres), Texas (1,100 acres), Puerto Rico (7,000 acres), and the US Virgin Islands (21,000 acres). In total, MosquitoMate requests 22,300 acres for 2019 and 26,800 acres for 2020, resulting in 49,100 acres across both years. The requested test locations and acres are listed in the applicant provided table (Table 1).

JUSTIFICATION FOR INCREASED ACREAGE AND TEST LOCATIONS

The stated purpose of this investigation is to continue to determine the pesticidal efficacy of a *W. pipientis* microbial pesticide for suppression and elimination of *Ae. aegypti* mosquito populations. The primary two justifications for the acreage and test locations are:

1. Justification for increased acreage in California is the need to test the hypothesis that larger test areas will mitigate the effects of immigration and improve suppression of *Aedes aegypti* populations. Previous trials in California found that a release rate of ~750 WB1 males/acre is adequate to suppress the *Ae. aegypti* population in this location. Testing in 2019 aims to determine if larger test areas allow for a lower release rate.
2. Justification for testing in Texas, Puerto Rico, and the US Virgin Islands is the need to test in locations with diverse climates and terrain.

EXPERIMENTAL DESIGN

Protocol. Generate efficacy data which will be used to support a FIFRA Section 3 registration. Including:

- a. Evaluate competitiveness and suppression of released males in the field in different climates and habitats.
- b. Test for hypothesized increased frequency of *Ae. aegypti* eggs failing to hatch.
- c. Test for a significant reduction in *Ae. aegypti* population size compared to one or more control sites.

Application Method. MosquitoMate provided a general protocol (Appendix A, MRID50860901) which outlined the experimental design that would be applied to all sites. Site specific design details were included in other portions of the Section G and will be discussed below in the “Site Specific Testing” section. MosquitoMate proposes to release wAlbB infected male mosquitoes multiple times weekly at treatment areas. Treatment areas will vary in size based on test location and experimental design.

A 7:1 ratio of *Ae. aegypti* males infected with wAlbB-strain to each wild *Ae. aegypti* male was shown to be needed to result in a population suppression effect. Mark-release-recapture studies and ongoing monitoring will be performed to determine the wild *Ae. aegypti* male population size to ensure that the minimum 7:1 ratio is obtained throughout the experiment. WB1 males will be released either by point release or continuous release from a moving ground vehicle. Differences in the two release methods is not expected to impact efficacy.

Treatment and control areas will be separated by three times the typical *Ae. aegypti* dispersal distance and will consist of similar characteristics (e.g., socioeconomic, human density, etc). Mosquito sampling will be done weekly using BG Sentinel traps, AGO traps, or similar traps, for the adult population and ovicups for eggs. Although the trap type may differ among test locations, the applicant will use the same trap type and trap density in treatment and control areas within a test location. Within test locations, the adult collection and ovicup egg collection data from the experimental site will be compared to the control site to examine for the effect of the release of the product on the native mosquito population. The applicant will also monitor mosquitoes at the release sites to verify the absence of wAlbB strain infected female *Ae. aegypti* mosquitoes.

In addition to field monitoring and testing, the applicant will also assess quality of shipped males. For adult WB1 males that are shipped, quality will be assessed periodically by monitoring survival/fitness over time and approximating shipment mortality at each release site. Specifically, the applicant will determine shipment mortality by comparing the mortality of shipped males released into a laboratory cage with that of a similar set of males that were not shipped.

For data analysis, Abbott's formula will be used to calculate percent reduction in the mean trap counts in the treated area, relative to the mean trap counts in the untreated area. To determine significance, data sets that are not normally distributed will be initially analyzed by non-parametric comparison and depending on the distribution of the data, additional analyses (e.g., parametric analyses) may be used (per response letter from registrant dated May 1, 2019). However, specific statistical tests need to be determined *a priori* based on the type of data intended to be collected and study design. *Post hoc* tests cannot be used to increase power for analysis. The applicant will also compare data between consecutive years at the same site. For example, in California, various comparisons can be made using data obtained in 2019, which will include treatment areas that were areas treated in 2018, areas that were used as untreated control areas in 2018, and areas that were not involved in the 2018 study.

Containment. Male wAlbB mosquitoes will be reared in EPA registered pesticide-producing establishments (i.e., Lexington, KY, South San Francisco, CA). If shipped, male mosquitoes or eggs will be in sealed shipping containers. Adult male mosquitoes will be transported to field sites, where they will be released immediately. At the end of the experiment, all equipment used in the rearing process will be frozen or heated to at least 100°C. For disposal, if males are not to be applied, they will be frozen to kill them or heated to 100°C, cooled, put in a Ziploc-type PE bag and disposed of with trash and/or garbage.

Site Specific Testing. Although the general protocol will be followed in each location, some site-specific experiments are proposed.

California. In addition to the data collected from the general protocol, experiments in California aim to: better understand pesticidal efficacy under a range of WB1 'doses', test efficacy in wide-area applications, and test whether local elimination is possible. Previous work in California found that ~750 males per week per acre was enough to suppress the local population across 724 acres. The applicant proposes to increase the acreage tested to mitigate 'edge effects' and to determine whether this would allow for a lower release rate. The applicant proposes four release rates: ~100 males/week/acre across 9,100 treated acres, ~150 males/week/acre across 2,700 treated acres, ~200 males/week/acre across 3,000 treated acres, and ~300 males/week/acre across 1,800 treated acres. The data gathered from these experiments will be used to inform a Section 3 label by permitting more accurate recommendations for a minimal release rate based on treated acres.

Texas. The addition of Texas as a test location provides the applicant with a site centrally-located in the USA. The applicant proposes to release up to 1,500 males per week per acre across 150

acres of treatment area (Figure 3). For 2020, two release areas, each up to 200 acres, have been requested.

Puerto Rico. The Puerto Rico site will provide the applicant data from a semi-tropical climate with varied terrain and high-density housing. In 2019, the applicant proposes a small scale (500 acres) mark-release-recapture experiment to assess the size of the wild population and measure male dispersal, which will inform release numbers during the subsequent 2020 trial. In 2020, the applicant proposes release rates of 300-700 males per week per acre across 3,000 treated acres (see Figure 3). The treatment area will be divided in up to 25 treatment areas with an additional ~3,000 acres designated as control. Treatment and control pairs will be randomized based on entomological and demographic parameters.

US Virgin Islands. The US Virgin Islands site will provide the applicant data from a tropical climate with a semi-urban landscape. Similar to Puerto Rico, the applicant proposes small scale (500 acres) mark-release-recapture experiments in 2019 to determine the wild population size and male dispersal. In 2020, the applicant proposes release rates of 300-700 males per week per acre across 10,000 treated acres (Figure 4). However, it is unclear why 10,000 control acres are necessary in the US Virgin Islands.

AMOUNT OF ACTIVE INGREDIENT

According to applicant provided Table 1 (listed below), up to 0.21oz of the active ingredient may be applied to the treatment acreage over the two-year test period.

DISCUSSION

BPPD has reviewed the experimental protocol (Section G) submitted with the application and found it to be **acceptable** pending the following changes:

- The amount of control acreage in Puerto Rico is reduced to 2,000 acres.
- The amount of control acreage in the US Virgin Islands is reduced to 2,000 acres.
- Replace sentence “When appropriate, additional analyses may be used to gain additional power...” with a sentence indicating that parametric analyses will be used when analyzing normally distributed data as described in MosquitoMate Inc’s initial response dated May 1, 2019 to the 75-day deficiencies letter.

The experimental protocol does not contain sufficient detail or justification to support all requested control acreage. The applicant states in the general protocol that “to offset the substantial cost of monitoring, untreated control areas can be smaller in size than the treatment areas...”. In the US Virgin Islands, it appears that 10,000 control acres in 2020 is significantly more than necessary. In California, 2000 control acres was deemed sufficient for testing, and it is unclear why this would not be sufficient in other locations as well. Therefore, BPPD suggests an 8,000-acre reduction in the amount of control acreage for the US Virgin Islands so that the total amount of control acres in 2020 is 2,000. The same logic is applied to Puerto Rico, resulting in 2,000 control acres in 2020 rather than the requested 3,000 acres. The reduction in 2020 control acres across sites results in an overall reduction of 9,000 acres. With this reduction, the total approved acreage for 2019-2020 is 40,100 acres (Table 2).

Table 1. Applicant provided table on acreage, number of mosquitoes, and amount of active ingredient to be released in 2019-2020.

Location	ACRES				NUMBER OF MOSQUITOES		WEIGHT OF MOSQUITOES (lbs)		AMOUNT OF ACTIVE INGREDIENT (OZ)	
	2019		2020							
State	Test	Control	Test	Control	2019	2020	2019	2020	2019	2020
California	18,000	2,000	-	-	98,400,000	-	304	-	4.9 x 10 ⁻²	-
Texas	150	150	400	400	4,000,000	11,000,000	12.3	34.0	2.0 x 10 ⁻³	5.4 x 10 ⁻³
Puerto Rico	500	500	3,000	3,000	200,000	156,000,000	0.62	481	9.9 x 10 ⁻⁵	7.7 x 10 ⁻²
USVI	500	500	10,000	10,000	100,000	156,000,000	0.31	481	4.9 x 10 ⁻⁵	7.7 x 10 ⁻²
Total Per year	19,150	3,150	13,400	13,400	102,700,000	323,000,000	317	997	5.1 x 10 ⁻²	1.6 x 10 ⁻¹
Total Area (Test and Control)	49,100				425,700,000		1,314		2.1 x 10 ⁻¹	

Weights are calculated as a 1.4g maximum mosquito weight and 0.014mg maximum AI weight for approximately 1,000 mosquitoes

Table 2. BPPD approved table on acreage, number of mosquitoes, and amount of active ingredient to be released in 2019-2020.

Location	ACRES				NUMBER OF MOSQUITOES		WEIGHT OF MOSQUITOES (lbs)		AMOUNT OF ACTIVE INGREDIENT (OZ)	
	2019		2020							
State	Test	Control	Test	Control	2019	2020	2019	2020	2019	2020
California	18,000	2,000	-	-	98,400,000	-	304	-	4.9 x 10 ⁻²	-
Texas	150	150	400	400	4,000,000	11,000,000	12.3	34.0	2.0 x 10 ⁻³	5.4 x 10 ⁻³
Puerto Rico	500	500	3,000	2,000	200,000	156,000,000	0.62	481	9.9 x 10 ⁻⁵	7.7 x 10 ⁻²
USVI	500	500	10,000	2,000	100,000	156,000,000	0.31	481	4.9 x 10 ⁻⁵	7.7 x 10 ⁻²
Total Per year	19,150	3,150	13,400	4,400	102,700,000	323,000,000	317	997	5.1 x 10 ⁻²	1.6 x 10 ⁻¹
Total Area (Test and Control)	40,100				425,700,000		1,314		2.1 x 10 ⁻¹	

Weights are calculated as a 1.4g maximum mosquito weight and 0.014mg maximum AI weight for approximately 1,000 mosquitoes

Figure 1. Applicant provided map of California treatment and control areas. Four proposed treatment areas are colored in blues and purple. Five control areas are highlighted in orange.

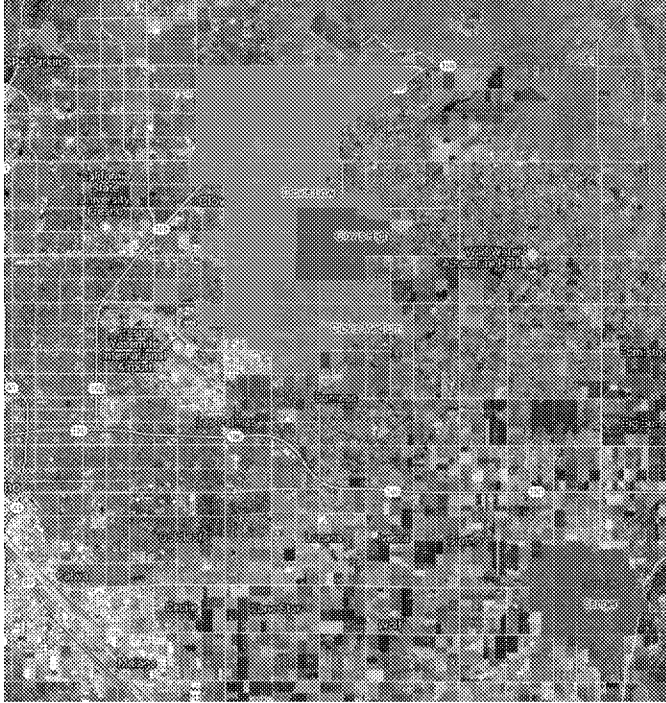
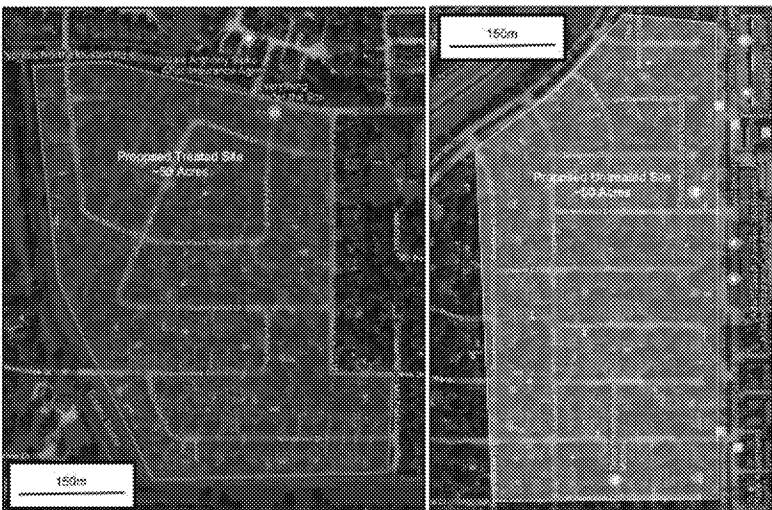


Figure 2. Applicant provided map of proposed Texas treatment (orange) and control (yellow) areas. The applicant plans to test across 150 acres of treatment area and 150 acres of control area.



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